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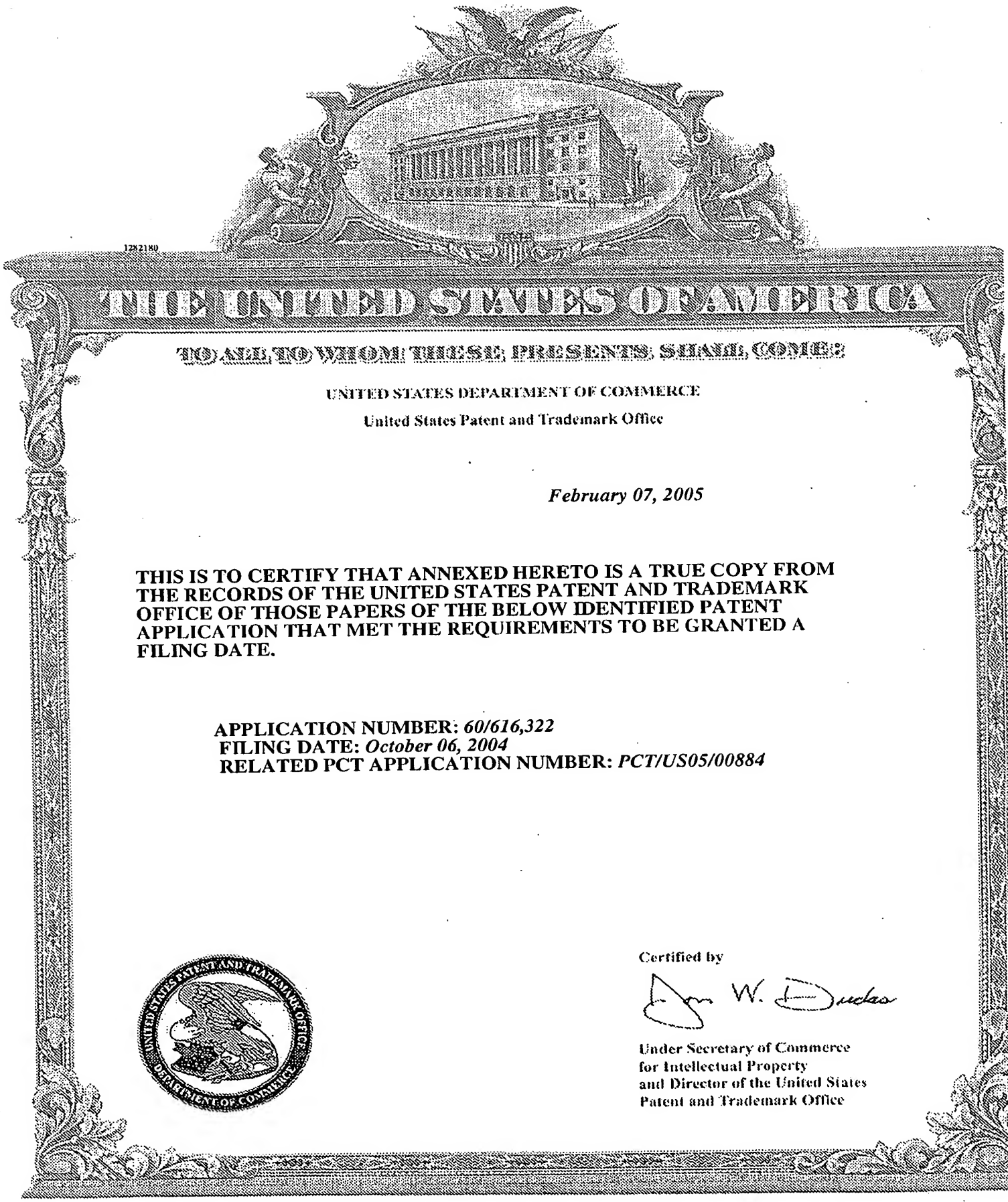

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This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(b)(2).

Docket Number: R3722	Type a plus sign (+) inside this box	+

Inventor(s)/Applicant(s)			
Last Name	First Name	Middle Initial	Residence (City and either State or Foreign Country)
Siegel	Steven	J	Berwyn, PA

Title of the Invention (280 Characters Maximum)

Long Term Implants of Risperidone

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Enclosed Application Parts (*check all that apply*)

[X] Specification *Number of pages:* 28 [ ] Small Entity Statement

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[ ] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature: Steven Siegel Date: 10/5/4

Typed or Printed Name: Steven Siegel

[ ] Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

PROVISIONAL APPLICATION SUBMISSION TO USPTO – CONTENTS PAGE

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First-named Inventor	:	Siegel
Submission Date	:	10/6/04
Prepared by	:	Matt Thomas

CONTENTS LISTED IN ORDER :

Total Number of Pages : 28

A. Research Plan

Statement of Hypothesis and Specific Aims

Rationale: This application responds to **PA-04-006 titled Neurotechnology Research, Development, and Enhancement,** which specifies "Delivery systems for exogenous agents such as drugs" among its goals. We address this goal by developing an implantable long-term delivery system to improve medication adherence in schizophrenia.

Medication nonadherence is the highest determinant of relapse in schizophrenia. Therefore, an intervention that helps patients remain on medication for extended periods would substantially improve clinical outcome. Industry has been reluctant to develop long-term delivery systems due to uncertainty in the regulatory process and patient acceptance. Barriers to development of long-term delivery systems in academia include access to appropriate compounds, high cost of Food and Drug Administration (FDA) quality testing and low value placed on treatment development for professional advancement. These issues are addressed in the current proposal. The investigators previously developed a prototype long-term delivery system using the typical antipsychotic agent haloperidol with poly-lactide-co-glycolide (PLGA). However, feedback from patients, families and physicians indicate that such a system would be more acceptable with a newer antipsychotic drug such as risperidone.

Release and safety characteristics from PLGA implants vary substantially for different agents. Therefore, design specifications for one compound can not be directly translated to another. The current proposal would enable the creation and testing of a biodegradable, implantable, long-term delivery system for the antipsychotic agent risperidone. Studies were designed after consulting with the FDA Psychopharmacology team and will conform to FDA guidelines in collaboration with the NIMH synthesis program. Completion of the proposed studies would allow investigators to seek investigational new drug approval for phase-I clinical trials in humans, provide sufficient evidence to justify industrial investment in this novel approach and improve patient care.

Each of the three interdependent aims accomplishes a mandatory component of the approval criteria to advance implants to humans. The first Aim meets the FDA-required demonstration that fabrication and storage of implants do not alter the molecular structure of constituent materials. The second Aim determines the *in-vivo* release profile for risperidone as a function of lactide to glycolide ratio and PLGA inherent viscosity. The final Aim will evaluate toxicological effects of a semiannual subcutaneous delivery system in two required species.

SPECIFIC AIMS

1. ***To assess the physical and chemical characteristics of risperidone as a candidate for biodegradable PLGA long-term delivery systems.*** Need: Drugs and polymers used in long-term delivery systems must fulfill a series of criteria to be considered appropriate for human use. These include physical and chemical stability during fabrication as well as during sequestration at body temperature over the delivery interval. Experimental Approach: We will assess the stability of risperidone during implant fabrication, storage and under *in-vitro* physiological conditions using High Pressure Liquid Chromatography. Additionally, we will assess the stability of proposed medical grade PLGA polymers during implant fabrication and storage using differential scanning calorimetry and inherent viscometry.

2. ***To determine in vivo release profiles for risperidone as a function of lactide to glycolide ratio and polymer inherent viscosity.*** Need: Release profiles for a fixed concentration of risperidone will vary as a function of polymer composition (lactide to glycolide ratio) and inherent viscosity. Modulation of these parameters will produce implants that provide controlled risperidone release over a desired interval. Experimental Approach: We will utilize Good Manufacturing Practices (GMP) to determine the relationship between polymer characteristics and risperidone release in rat. Ten polymers will be tested to determine a combination of individual polymers to provide six months of steady risperidone release.

3. ***To assess local tissue and systemic toxicity for a semiannual formulation of implantable PLGA-risperidone implants.*** Need: Risperidone-PLGA implants will need to demonstrate safe, consistent release in two species (rodent, non-rodent) prior to moving to phase-I human trials. The final aim will demonstrate a lack of local tissue irritation or systemic toxicity following chronic *in-vivo* release of risperidone from PLGA implants in rat and dog. Experimental Approach: Three doses of the PLGA-risperidone implant system derived in Aim 2 will be placed in the subcutaneous space in rat and dog. Animals will be sacrificed at 6 or 12 months for histology. Blood will be taken every 2 weeks for serum risperidone levels and monthly for hematology and serum chemistry.

B. Background and Significance:

B. 1. Need for improved medication adherence: There are multiple causes of medication nonadherence in schizophrenia, ranging from adverse side effects to lack of family support.(Robinson et al., 2002) Nonadherence with medication has been identified as a major risk factor for relapse and rehospitalization with 55% of patients displaying significant difficulties adhering to treatment recommendations.(Corriss et al., 1999) Nonadherent patients are twice as likely to undergo rehospitalization from relapse, resulting in a poor quality of life.(Svarstad et al., 2001) For example, approximately 50% of treated schizophrenia patients relapse within one year of their latest episode, spending 15–20% of their time in psychiatric institutions.(Ayuso-Gutierrez and del Rio Vega, 1997) Furthermore, the economic and social burden of healthcare costs for nonadherence in mental illnesses is estimated at $2.3 billion annually.(Menzin et al., 2003) Additional studies indicate that an actual medication adherence rate of 23% is in stark contrast to the patient-reported rate of 55%, while others find as few as 12% of patients achieve one year of uninterrupted antipsychotic medication.(McCombs et al., 1999; Velligan et al., 2003) Although many studies report medication adherence rates of 50%, it is increasingly thought that such data may be skewed, as many patients cease medication treatment once outside clinical trials.(Seeman, 2001) However, once a physician and patient reach consensus regarding implantable medication, the treatment plan could be rapidly implemented using a 15 minute outpatient visit. Based on the design outlined below, this would require only one procedure every six months, rather than the 6-12 necessary for depot injections over this interval using either monthly haloperidol decanoate or biweekly risperidone Consta® (Janssen Pharmaceutica). Consequently, it is important to note that although implants would provide improved convenience and access to medication, they would not replace the need for patients to interact with psychiatrists and therapists. Rather, improved adherence and continuous access to medication would allow patients and physicians to focus on issues other than medication adherence in the service of a more comprehensive treatment plan.

B. 2. Use of depot preparations: Previous approaches to improve adherence through parenteral administration have proven effective. Depot injections work well to deliver antipsychotic drugs over several weeks with enhanced efficacy over oral dosing.(Adams et al., 2001) However, limitations of depot formulations restrict more significant improvements in adherence and efficacy. For example, adverse effects are occasionally manifest and must be endured for the remainder of the treatment interval due to the inability to remove depot formulations. Prolonged pain often persists at repeated injection sites leading many patients to cease treatment.(Kane et al., 1998) Decanoate formulations are further limited by chemistry, with many compounds unable to form the ester linkage needed to make pro-drug molecules. Recently, PLGA microspheres have been used to administer a biweekly formulation of risperidone that does not require formation of a pro-drug (Risperidone Consta®, Janssen Pharmaceutica). Although this approach shows promise in controlled studies, injections must be administered biweekly requiring 24 annual treatment decisions.(Martin et al., 2003; Harrison and Goa, 2004) Despite early promise, it is not yet clear what effect such a short delivery interval will have on practical application of this technology since adherence rates in the real world are estimated to be substantially lower than those in well-funded, labor intensive clinical trials.(Visco et al., 1999) Thus, depot medications may not provide adequate sustained care to many patients.

B. 3. Indications to add availability of implants: To address the need for more sustained treatment adherence, new methods of long-term medication delivery have been investigated using implantable systems for the treatment of schizophrenia.(Siegel et al., 2002) Implantable systems have the capability to optimize a medication's therapeutic properties, rendering treatments that are more safe, efficacious and reliable.(Dash and Cudworth, 1998) Less medication is generally required and side effects can be minimized. Implantable systems can also be removed by a physician in case of adverse side effects, offering a degree of reversibility not available with depot injections. Additionally, mandatory removal at the end of the delivery interval is eliminated with biodegradable systems, cutting in half the inherent invasiveness to the patient.(Fischel-Ghodsian and Newton, 1993)

B. 4. Merits of PLGA biodegradable polymers: Several existing biodegradable implant systems rely on PLGA polymers which are highly biocompatible and physically strong.(Kitchell and Wise, 1985) The degradation products of PLGA, lactic acid and glycolic acid, are water soluble, non-toxic products of normal metabolism that are either excreted or further metabolized to carbon dioxide and water in the Krebs cycle.(Curtis, 1983; Okada and Toguchi, 1995) A limited number of systems already utilize PLGA and other biocompatible polymers to successfully achieve long term delivery, including devices to deliver thyrotropin-releasing hormone, L-dopa to treat Parkinson's Disease and naltrexone microspheres in treating narcotic addiction.(Sharon and Wise, 1981; Sabel et al., 1990; Okada and Toguchi, 1995) Intraocular systems, including Vitrasert® (Bausch and Lomb), offer similar PLGA-based biocompatible delivery systems with controlled-release drug therapy for periods ranging from several days up to one year.

B. 5. Drug-polymer interactions: Drug release profiles are not constant for a given polymer. Rather, release is dependent on the interaction of each pharmaceutical agent with the polymer. Although the proposed implantable platform is more flexible than decanoate formulations in allowing incorporation of a broader range of compounds, each medication must be individually examined in light of its unique interaction with the polymer

matrix and therapeutic objectives. Indeed, one limitation of PLGA systems has been the observation that many drugs result in an unfavorable pattern of slow initial release (lag) followed by a period of rapid release (dumping). Many factors affect these patterns of medication release from polymeric systems including drug diffusion rate, drug/polymer binding, pH, source/sink concentrations and implant hydration. Furthermore, lag to initial release and drug dumping could be caused by distinct reaction rates for hydration of implants, polymer degradation and drug diffusion through the polymer matrix, highlighting the need to analyze each drug-polymer combination independently. We have shown that haloperidol and risperidone (preliminary data) yield favorable release profiles lacking a distinct period of drug dumping, suggesting that they are appropriate candidates for long-term release from PLGA implants.(Siegel et al., 2002)

B. 6. Ethical considerations: In addition to the scientific challenges involved in engineering a long-term medication delivery system, ethical issues related to the use of invasive interventions among psychiatric populations must be considered. For example, patients will need to demonstrate capacity to consent for the use of implants. A recent study to assess comprehension and attitudes towards implantable psychiatric medication indicated that 88% of patients were able to accurately comprehend the procedure, risks and benefits of implantable medication.(Irani et al., 2004, appendix) This study also indicated that a small subset of patients that could not demonstrate clear comprehension of the issues involved in long-term delivery systems were least likely to accept such an intervention for themselves. Of note, 42 % of patients in that cohort indicated that they would be likely to accept an implant to receive their medication if such a system were available. Additionally, a subsequent survey of 450 family members indicates that 78% of care givers support the use of implants for their mentally ill relative. (manuscript in preparation). Despite reports suggesting that many patients and their families would accept implants, concerns have been raised regarding the potential for coercing patients who might otherwise not accept implants. Although ethical issues are beyond the scope of this application, the investigators are committed to proceeding in an ethically and clinically responsible fashion. For example, the principal investigator recently organized a symposium to discuss ethical concerns related to implants. This public discussion included representatives from diverse perspectives including The Center for Biomedical Ethics at the University of Pennsylvania, The Food and Drug Administration (FDA), National Association for the Mentally Ill, The Treatment Advocacy Center, The Pennsylvania Mental Health Consumers Association and The Mental Health Association of Southeastern Pennsylvania. Efforts to incorporate patient, family and community concerns will continue under the auspices of a Center Grant from the Stanley Medical Research Institute (SJS, Principal Investigator). This Stanley Center Grant will also continue to provide much of the infrastructure needed to support the specific projects within this application as well as enabling investigators to develop complementary technologies and incorporate other pharmaceutical agents in the future. Thus, this application seeks to create a cooperative effort with related funding from a private patient advocacy agency and the NIH in the service of advancing a novel treatment strategy that could lead to future investment by industry.

B. 7. Summary: We advocate the availability of long-term implantable antipsychotic formulations for improved adherence in psychotic disorders. Preliminary data demonstrate biological feasibility of such a system with haloperidol for up to 14 months in monkey and rabbit. Furthermore, preliminary data demonstrate similar potential using the atypical agent risperidone *in-vitro*. Studies in the current proposal will provide

FDA-quality pre-clinical trials that would allow progression of risperidone implants from an academic exercise to a practical intervention in phase I clinical trials. In doing so, this application will advance the treatment of schizophrenia by empowering a novel treatment strategy to rapidly improve medication adherence with resulting improvement is clinical outcomes.

C. Preliminary Data:

C. 1. Investigators' Background: The principal investigator began this line of research in 1998 and has accumulated 6 years of experience with biodegradable polymers and long-term drug delivery. Dr. Karen Winey has been a close collaborator in this research for the past 5 years, bringing considerable knowledge of polymer chemistry and polymer engineering. As such, this team has significant background, knowledge and experience with clinical psychiatry, psychopharmacology and polymer material science. Preliminary data demonstrate (1) *in-vivo* proof of concept for biodegradable haloperidol implants of variable PLGA composition and (2) *in-vitro* extension of this approach to risperidone. Results suggest that implantable antipsychotic systems can achieve medication release within one week and could be tailored to last 12 months or more. Furthermore, procedures for implantation can be preformed in 15 minutes in the outpatient setting and could be easily tolerated with local anesthetic before subcutaneous placement. The current application will extend the investigators previous studies with haloperidol to create a long-term delivery system for the newer antipsychotic agent risperidone.

C. 2. Initial proof of concept with Haloperidol: Haloperidol was chosen as the initial drug for proof of concept based on the following five criteria: availability at low cost, high potency, chemical stability, demonstrated parenteral efficacy and tolerability in intramuscular and depot formulations. High potency agents such as haloperidol and risperidone are ideally suited for implant fabrication because only minute amounts of total drug are needed to sustain long-term release yielding small, easily tolerable implants. The first phase of development included demonstration of *in-vitro* release from a series of PLGA copolymers.(Siegel et al., 2002, see appendix) Formulation of drug and polymer implants was designed without surfactants or emulsifiers, instead using solvent casting from acetone followed by compression molding to yield a highly reproducible and simple method for fabrication. Acetone was chosen as an FDA Class III solvent (low toxicity with minimal need for removal of residual solvent) in which both haloperidol and PLGA polymers were soluble at greater than 100 mg/ml. Initial studies in rodents also demonstrated tolerability and bioactivity following implantation in mouse and rat for 3 weeks to 3 months.(Siegel et al., 2002) Subsequent studies focused on *in-vivo* evaluations of various drug polymer combinations in order to design semiannual implants.

C. 3. In-vivo release of haloperidol in monkey: Studies to evaluate annual haloperidol delivery in monkey were performed in collaboration with Dr. David Lewis at The University of Pittsburgh as part of ongoing research that required 1 year of continuous haloperidol in primates.(Lewis et al., 2001a; Dorph-Petersen et al., 2004) At that time, Dr. Lewis and colleagues were evaluating the effects of long-term haloperidol exposure on cortical anatomy in primates as a control for antipsychotic exposure in post mortem human schizophrenia studies. As such, the ability to provide monkeys with annual implants was seen as a relatively benign method in contrast to repeated injection for 12 months. This study demonstrated 14 months of haloperidol release in monkey (details follow).

C. 3. a. Subjects: The Institutional Animal Care and Use Committee at the University of Pittsburgh approved all protocols. Two monkeys (Macaca fascicularis, Rangos Research Facility) received implants. One animal received control implants with no drug, while the other received implants with 40% haloperidol by mass. Haloperidol (Sigma, St. Louis, MO) dosing was approximately 1 mg/kg/day over 12 months to achieve a serum concentration of 2-10 ng/ml. Each implant was made from a single polymer of PLGA in ratios of 75:25, 85:15, 90:10 (high and low inherent viscosity), 95:5 and 100:0 (Medisorb®Alkermes, Cincinnati, OH).

C. 3. b. Implant fabrication: Polymers and haloperidol were mixed in a proportion of 60/40 by mass and solvent cast from acetone (Fisher Scientific, Pittsburgh, PA). The resulting film was compression molded to disk-shaped implants of 20 mm diameter with average thickness of 1.22 ± 0.0 mm, mass of 493 ± 2 mg and density of 1.28 ± 0.0 g/cc.

C. 3. c. Pharmacokinetic determination: Blood was drawn twice per month. Specimens were separated by centrifugation and haloperidol levels assayed by high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection (Figure 1). Assays from control animals yielded no drug detected.

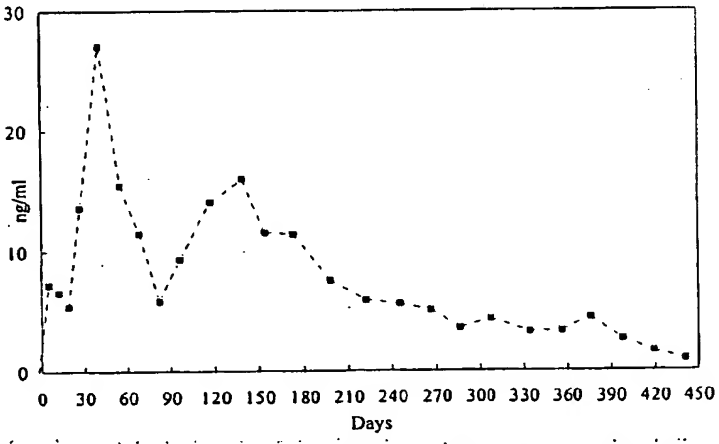


Figure 1: Haloperidol release occurred for a total of 443 days. The average serum concentration was 10.5±1.5 ng/ml during the first 224 days. One value was higher than the rest, with a maximum level of 27.1 ng/ml on day 40. During the subsequent 176 days, serum haloperidol level was sustained at lower concentration before the end of release. Mean concentration during this period was 4.0±0.4 ng/ml. Release decreased during the last 45 days of the study with a mean serum level of 1.2±0.3 ng/ml.

C. 4. Haloperidol release in Rabbit: Implants in rabbits were tethered to assist in locating implant sites at necropsy. Two different implant systems were tested in rabbit. One design contained an amalgam of five different polymers to affirm previous findings in monkey. The other design was composed of a single long-lasting polymer. The aim of the single polymer model was to reduce the initial spike while maintaining release for one year. These studies demonstrated haloperidol release for 13 months in rabbit (details follow).

C. 4. a. Subjects: Rabbits (N=12, Covance, Denver, PA) ranged from 4.0 to 5.7 kg. Five animals received implants composed of a single polymer, 100% PLA, with a 40% haloperidol load for an average dose of 418±7 mg/kg yielding a daily dose of 1.13±0.02 mg/kg/day for anticipated delivery of 365 days. Five additional animals each received implants of a combined polymer system including 75:25, 85:15, 90:10 high inherent viscosity, 90:10 low inherent viscosity and 100:0 PLGA. The average dose in this group was 473±4 mg/kg with an expected delivery of 365 days yielding an average daily dose of 1.29±0.03 mg/kg/day. Two rabbits received implants without drug as a control. One control received 100% PLA implants to mimic the single polymer condition, while the other

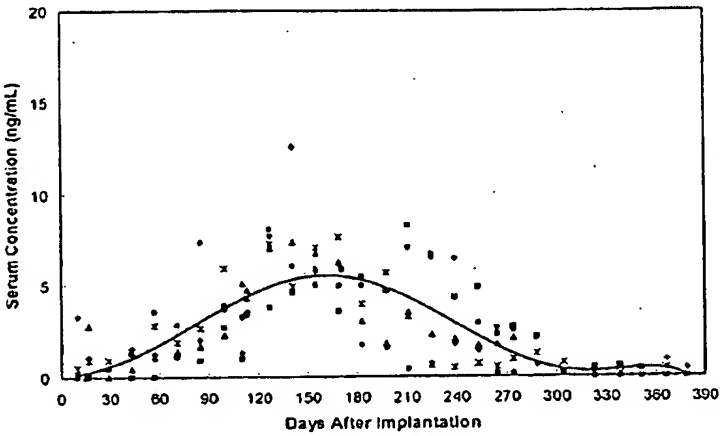
received implants composed of 75:25, 85:15, 90:10 high inherent viscosity, 90:10 low inherent viscosity and 100:0 PLGA to mirror the combined polymer system.

C. 4. b. Implant fabrication: Implants for rabbits were made using procedures described for monkeys with an average mass of 536±2 mg and density of 1.24±0.00 g/cc.

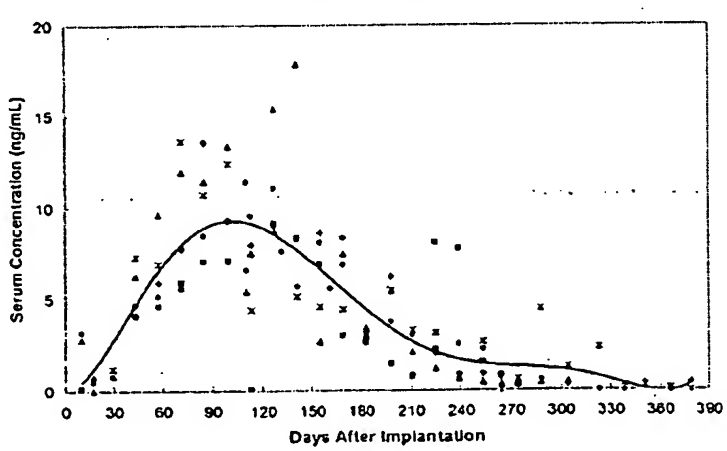
C. 4. c. Pharmacokinetic determination: Blood samples were drawn twice per month. Serum haloperidol was determined by HPLC with UV detection following solid phase extraction (Oasis MCX columns, Waters, Milford, MA) (Figure 2).

Figure 2: Haloperidol release from polymer implants in rabbit. Each chart displays data from five animals (circle, square, . triangle, diamond and asterisk).

(A) Five rabbits with the single polymer system showed release for 379 days. Mean serum haloperidol concentration was 2.5±0.4 ng/ml for that duration. Between days 57 and 274, mean concentration was 3.8±0.4 ng/ml. The highest point of release occurred on post-operative day 141, with 12.6 ng/ml in one animal.



(B) Five rabbits with the combination polymer system showed haloperidol release for 379 days. Mean serum level was 4.0±0.6 ng/ml during that period. Release in combination animals is represented by a two-phase profile similar to that observed in monkey. A period of larger initial release occurred during the first 198 days with mean haloperidol serum level of 6.1±0.7 ng/ml (maximum, 17.8 ng/ml, day 141). Levels then tapered for the remaining 181 days with a mean level of 1.1±0.3 ng/ml.



C. 4. d. Histopathology: Five rabbits were sacrificed after nine months to obtain interim pathological analyses. The remaining seven rabbits were sacrificed after an additional four months of study. Remains of implants were found tethered in place upon sacrifice in all animals except controls. HPLC/UV and NMR spectroscopy confirmed the presence of haloperidol and PLGA breakdown products in residual implants. Histological analyses showed all organ systems in control and treated rabbits were within normal limits.

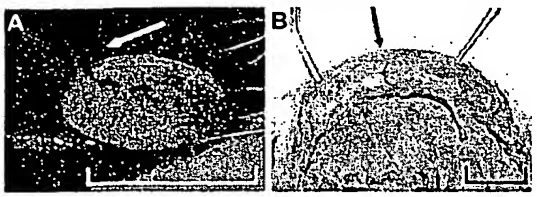


Figure 3: (A) Placement of implants during rabbit surgery. A tethered implant (white arrow) is shown. The incision was enlarged to enable a photograph. (B) Necropsy in rabbits showing a degraded implant (black arrow) at the tethering location between two hemostats. No fibrosis was observed upon implant removal. Scale bar = 20 mm in both images

C. 5. Indications to change to Risperidone: Despite its feasibility for implants, haloperidol is viewed by many in the psychiatric community with reservation. However, newer agents such as risperidone are seen as relatively benign with respect to side effects, while offering similar efficacy. Sources including patients, family members, and healthcare providers, as well as discussions during research symposia, peer-reviewed manuscripts and NIMH program officers all report that risperidone is considered superior to haloperidol with regard to risks for long-term side effects. Additionally, several studies have extolled the virtues of newer agents.(Kane, 1999; Lewis et al., 2001b; Karow and Naber, 2002; Csernansky, 2003; Davis et al., 2003; Keith and Kane, 2003; Lambert and Castle, 2003; Sharma and Antonova, 2003; Strakowski et al., 2003; Keefe et al., 2004) Taking all of these issues into consideration, the investigators believe that long-term implants are more likely to become a reality if they are made with the newer agent risperidone. Of note, the major barrier to such a program in the past was limited access to adequate quantities and high cost ($7000 per gram) of risperidone. However, the current application is enabled through collaboration with the NIMH synthesis program which will supply risperidone. Therefore, studies in the proposed R-01 will incorporate the atypical antipsychotic medication risperidone in long-term biodegradable implants.

C. 5. a. Stability of Risperidone: Preliminary studies related to Aim 1 evaluated the long-term stability of risperidone in physiological solution. Risperidone (10 mg) was dissolved in 100 µl of acetonitrile for subsequent dissolution in 1,000 ml of phosphate buffered saline, pH 7.0 (0.9 % NaCl, 0.01 M NaOH, 0.01 M NaH2PO4) to yield a final solution of 10,000 ng/ml. Risperidone solution was then stored in a light-safe amber bottle at 37°C and shaken at 40 revolutions per minute. A 1-ml sample was taken three times per week and examined by UV spectroscopy (Amersham Biosciences, Buckinghamshire, UK) for drug content. Analyses indicate that the concentration of drug remained stable with an overall decrement of 0.12% over 285 days thus far, equivalent to 0.0005% per day (linear trendline: y = -0.00004x + 9.4774). The positive control trendline for the subsequent in-vitro study of polymer degradation is displayed in Figure 4 and indicates similar stability during the first 2 months of testing (in progress).

C. 5. b. In-vitro risperidone release from individual polymers: Risperidone release profiles from three polymers were evaluated in preparation for Aim 2. Implants were fabricated as previously described with 20% risperidone (RBI, Flanders, NJ) and 80% polymer (Medisorb®, Alkermes Inc. Cincinnati, OH) by mass. Three drug loaded implants were placed in separate light-safe bottles of phosphate-buffered saline on a shaker (500 ml, 37°C, 40 rpm). Aliquots of 1 ml were taken from each bottle three times per week and analyzed by UV spectrophotometry, after which 1 ml of buffer was reintroduced to maintain constant volume. *In-vitro* risperidone release profiles for 3 polymers are represented in Figure 4.

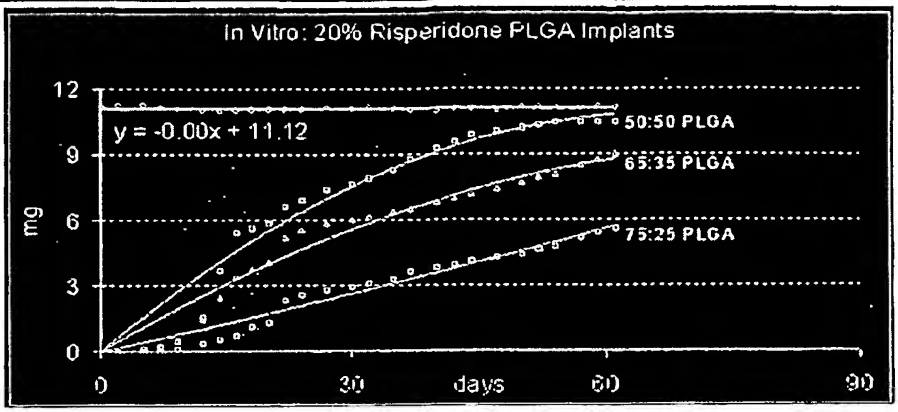
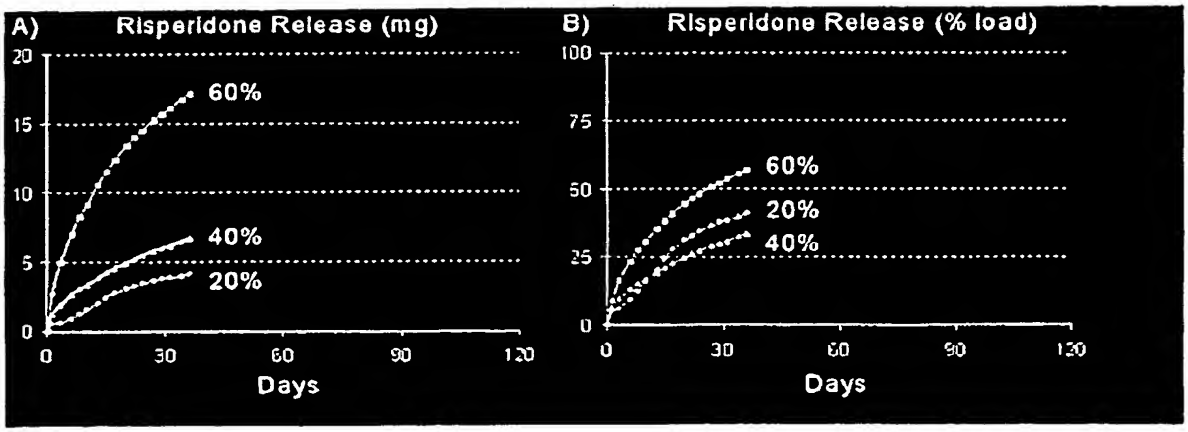


Figure 4: *In-vitro* cumulative risperidone release from implants containing either 50:50 High IV, 65:35 Low IV, or 75:25 High IV PLGA with 20% drug load. Data expressed as total mass of risperidone released over time. Each point represents a triplicate average with trendline to illustrate pattern.

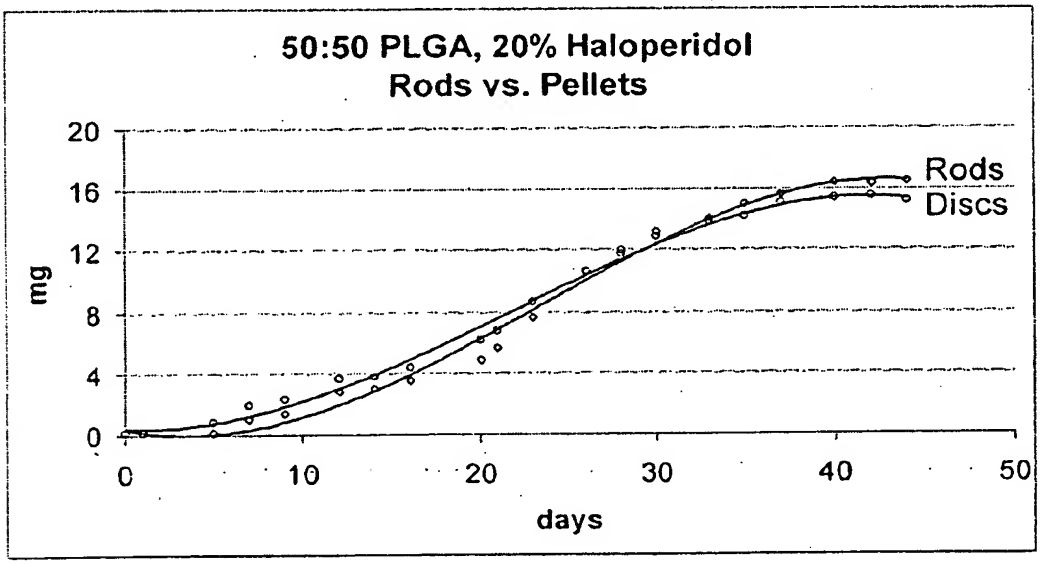
C. 5. c. Determination of maximum risperidone drug load: Studies are underway to examine the effects of drug concentration on risperidone release profile. The purpose of these studies is to establish the maximum concentration of risperidone that can be used in implants. Subsequent experiments in this proposal will utilize the maximum concentration so as to make implants as small as possible and therefore more easily tolerated. These studies began in August, 2004 and will proceed under the auspices of an intramural grant from the Institute for Medicine and Engineering at The University of Pennsylvania to Drs. Siegel and Winey to support preliminary studies for this application. Implants were prepared using a single polymer (85:15 PLGA High IV) combined with risperidone at ratios of 20%, 40% or 60% drug by weight (Figure 5). This polymer has an anticipated degradation interval of 4 months. Each implant has a mass of approximately 50 mg, yielding drug mass of 10, 20 and 30 mg respectively for the 20%, 40% and 60% drug load conditions. These studies will be replicated and additional percentages will be tested prior to the proposed start date for the current application.

Figure 5) Cumulative risperidone release from implants containing 85:15 PLGA with 20, 40 or 60% drug load by weight. Each point represents the mean of three replicates with a trendline to demonstrate overall pattern of release. **A)** Release expressed in total mg of risperidone from each 50 mg implant. **B)** Release expressed as a percentage of the amount of drug in each type of implant.



C. 5. d. Modification of Implant shape: Disc shaped implants were initially used to avoid complications related to intramuscular placement.(Klavon and Grubb, 1990; Townsend, 1991; Sarma and Hatcher, 1995) Subcutaneous tethers were then added to aid in localization during necropsy. However, this design necessitated a large incision, decreasing applicability to human. Therefore, we modified fabrication to produce rod shaped implants that can be inserted through a 4 mm hole. Of note, rigid PLGA implants obviate the need for a tool to guide implants under the skin, reducing risk of intramuscular placement. Preliminary studies then examined the effect of implant geometry on haloperidol release. Haloperidol (40%) was combined with 50:50 PLGA (60%) by solvent casting. Material was then compression molded into discs or slowly extruded into rods using a high pressure piston extruder (DACA Instruments, Goleta, CA). Release profiles from these two geometries are compared in figure 6. Future studies will use rod shaped implants.

Figure 6: Cumulative *in-vitro* release from disc and rod shaped implants. Each point represents the mean for three replicates of discs or rods. Each implant was placed in 500 ml of phosphate buffered saline, pH 7.0 at 37°C at 40 rpm in a the dark. Rods and disc were matched for weight. Release profiles are similar for both geometries.

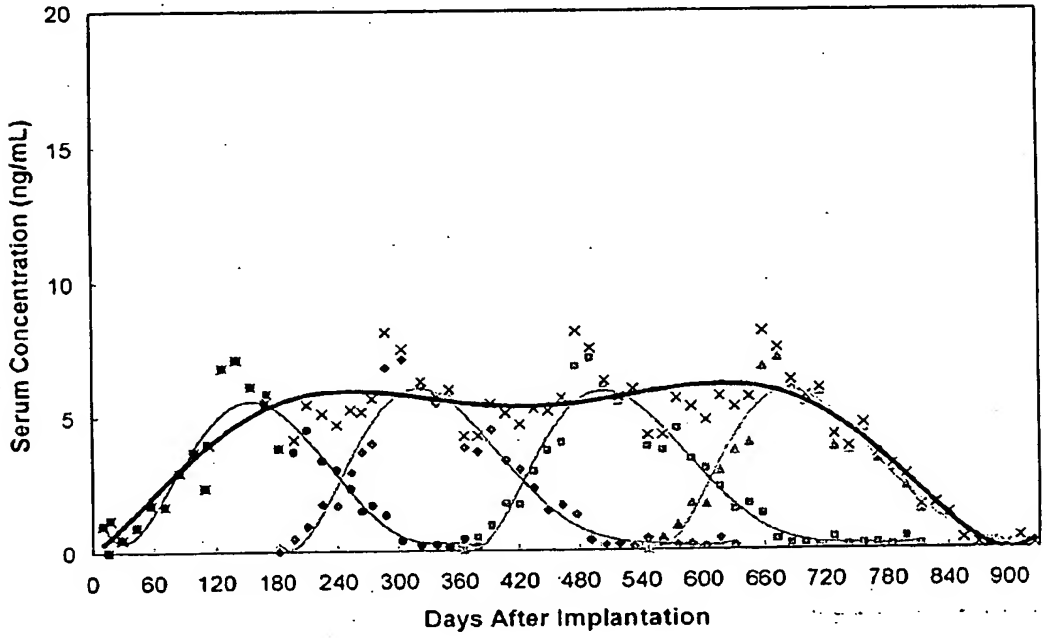


C. 6. Interpretation of Preliminary Data: Preliminary studies demonstrate *in vitro* and *in-vivo* proof of concept for long-term medication delivery in the treatment of schizophrenia. *In vivo* onset was rapid and serum concentration were within the target range of 2-15 ng/ml for a substantial portion of the release interval.(de Oliveira et al., 1996; Kapur et al., 1997; Foster and Goa, 1998) In the multiple polymer design, higher serum levels were apparent in the first seven months in comparison to the subsequent seven months (Figures 1 and 2b). It is likely that implants made from faster-degrading polymers such as 75:25 PLGA contributed to a larger initial phase of release. Therefore, the mass of 75:25 PLGA implants could be reduced in future studies to achieve lower initial dosing.

C. 6. a. Gaussian Release profiles: Release profiles from the single-polymer system in rabbit (Figure 2a) suggest that biodegradable implants could deliver

antipsychotic medication for 12 months. While this design avoided early periods of high serum concentration, the Gaussian pattern of release resulted in a 3-month lag to achieve target serum concentration. However, the symmetrical nature of the release profile opens the possibility of using overlapping implantations every six months to sustain drug delivery indefinitely (Figure 6). The goal of this approach will be to offset the steady decline from one set of implants with the gradual onset from a subsequent set. This concept is further developed in the Research Design and Methods of Aim 2 (Figure 7, page 29).

Figure 6: Model for continuous delivery from biodegradable implants. The release profile from 100% PLA implants in rabbits (Figure 2a) is superimposed temporally at six month intervals (gray trendlines) to model implantation intervals. Projected serum levels are summed at each time point, resulting in an anticipated total release plot (Xs). The black trendline models total release data. The system is projected to oscillate around a target serum level as long as implantations occur every 6 months.



C. 6. b. Scaling to Human: It is also important to note that there are considerable interspecies differences in the metabolism of many drugs, including haloperidol, with both rabbits and monkeys requiring approximately 15 to 30 fold higher doses than humans for equivalent plasma concentrations.(Bacopoulos et al., 1980; Jibiki et al., 1993; Klintenberg et al., 2002) Thus, the absolute doses used in preliminary studies approximate the amount of drug needed for a human, despite the difference in body mass. Since humans require approximately 1 mg/kg/month of haloperidol when given as a depot preparation, an implant system containing 600 mg would provide one year of treatment for a 50 kg patient. Thus, the implant design used in preliminary studies would necessitate only 1.5 gm of material with 40% drug load for one year of medication. Although preliminary *in vivo* studies utilized haloperidol, other high potency agents such as risperidone show promise for this approach. *In-vitro* release profiles for risperidone are promising, indicating that *in-vivo* systems could attain long-term delivery for six months or more. Dosing of risperidone implants is discussed further on page 31 in the Research Design and Methods for Aim 2.

D. Research Design and Methods:

The purpose of this application is to create an implantable long-term delivery system for the atypical antipsychotic medication risperidone. All methods have been designed to conform to FDA standards following consultation with members of the FDA Psychopharmacology team. Each Aim addresses specific goals or concerns that were raised by the FDA and are intended to provide the basis for an application for phase I human trials in the future. **Aim 1** will verify the physical stability of risperidone and ten proposed polymers during fabrication, storage and delivery interval. **Aim 2** will determine the *in vivo* risperidone release profile as a function of polymer composition and inherent viscosity in order to create a composite system capable of delivering steady state release for 6-months. **Aim 3** will then determine the pharmacokinetic profile of a composite delivery system while assessing local and systemic toxicity of the selected PLGA-risperidone implant formulation in rat and dog.

D. 1. Aim 1: To assess the physical and chemical characteristics of risperidone as a candidate for biodegradable PLGA long-term delivery systems. Rationale: Pharmaceutical agents and biodegradable polymers used in long-term delivery systems must meet a series of criteria in order to be safe for human use. These include physical/chemical stability during fabrication and storage, as well as stability of the drug during sequestration at body temperature during the delivery interval. Therefore, we will assess the stability of risperidone during implant fabrication, storage and under *in-vitro* physiological conditions. The stability of risperidone will be monitored using HPLC/UV analyses. Additionally, we will assess the stability and degradation of ten medical grade PLGA polymers during fabrication and storage using a viscometer to monitor changes in inherent viscosity and differential scanning calorimetry to determine if there have been any alterations in the glass transition temperature for each polymer.

D. 1. a. Design:

D. 1. a. i. Stability of risperidone: The stability of risperidone will be evaluated following solvent casting from acetone and slow extrusion molding to ensure that the compound can withstand processing into implants. Subsequently, risperidone content will be examined in a series of implants that are stored at 4°C for various intervals ranging up to 1 year to assess shelf life of polymer implants. Preliminary data indicate that the mass of risperidone is stable in a physiologic aqueous environment (37°C, phosphate buffered 0.9% saline, pH 7.0) for greater than 10 months using UV spectroscopy (page 8). Studies in Aim 1 will reproduce and extend this analysis for 12 months using GMP protocols for HPLC/UV detection to confirm this finding with a more selective technique. **Expected outcomes:** We anticipate that risperidone will retain its characteristic HPLC retention time and mass following solvent casting and compression molding. We also anticipate that risperidone will display less than 1% degradation during storage for 1 year. Based on preliminary data, we also predict that risperidone will display less than 1% degradation in a physiological, aqueous environment over 1-year as assessed with quantitative HPLC/UV detection.

D. 1. a. ii. Stability of PLGA polymers: In addition to evaluating the active pharmaceutical compound, we will determine the stability of ten proposed PLGA polymers during fabrication and storage. These studies will measure inherent viscosity and glass transition temperature as indicants of molecular weight distribution throughout the

fabrication and storage processes. **Expected Outcomes:** We predict that selected PLGA polymers will display less than 5% change in inherent viscosity (limit of accuracy for the procedure) and less than 2°C change in glass transition temperature following solvent casting and extrusion molding. Similarly, we predict that selected PLGA polymers will demonstrate less than 5% change in inherent viscosity and less than a 2°C change in glass transition temperature during storage in a dry environment at 4°C for 1 year.

D. 1. b. Methods:

D. 1. b. i. Materials: Risperidone will be synthesized using GMP methods and supplied by the NIMH synthesis program as specified in the letter of support from Jamie Driscoll, Chief of Research Services, Division of Neuroscience and Basic Behavioral Science. Medisorb® medical-grade PLGA polymers will be purchased from Alkermes Inc. (Cincinnati, OH) (Table 1). Acetone and all buffer reagents will be supplied by Sigma Chemicals (Cincinnati, OH). All material data safety sheets and certificates of analysis will be stored in a dedicated study binder and will be available for inspection by the FDA.

Table 1: Polymers from Alkermes are listed with the range of degradation intervals that will be used for studies in this proposal. All selected polymers are amorphous and therefore lack a distinct melting point. Abbreviations: H – High inherent viscosity (0.66-0.80 DL/g), L – Low inherent viscosity (0.50-0.65 DL/g)

PLGA Polymers	Formulation	Glass transition °C	Degradation interval
1. 50:50 L	50% d,l-lactide	50-55	1-2 months
2. 50:50 H	50% glycolide		
3. 65:35 L	65% d,l-lactide	45-50	3-4 months
4. 65:35 H	35% glycolide		
5. 75:25 L	75% d,l-lactide	48-53	4-5 months
6. 75:25 H	25% glycolide		
7. 85:15 L	85% d,l-lactide	50-55	5-6 months
8. 85:15 H	15% glycolide		
9. 100:0 L	100%	50-55	12-16 months
10. 100:0 H	d,l-lactide		

***D. 1. b. ii. 1. Temporal Stability of Risperidone in physiologic solution*:** Three replicates of risperidone solution will be prepared as follows. Risperidone (10 mg) will be dissolved in 100 µl of acetonitrile for subsequent dissolution in 1,000 ml of phosphate buffered saline (pH 7.0, 0.9 % NaCl, 0.01 M NaOH, 0.01 M NaH2PO4) to yield a final solution with a concentration of 10,000 ng/ml. Risperidone solutions will then be stored in light-safe amber bottles at 37°C. Three aliquots (100 µl) of each sample will be drawn at monthly intervals for HPLC analysis. Each 100 µl aliquot will be diluted with 900 µl of sample buffer to a final concentration of 1000 ng/ml and three replicates of 50 µl (mass = 50 ng) will be injected for a HPLC detection. The concentration of risperidone will then be plotted across time and the slope of the resulting line will reflect stability of the compound. Each time point will also include a series of three negative control samples. A new standard curve will be prepared with each run to control for slight variation in retention time. HPLC will be performed using a Waters 1525 binary pump, 717 autosampler with 2487 dual wavelength UV detector and Breeze® analysis software and Waters XTerra MS C18 5um, 4.6X150 mm column. **Mobile phase** will consist of 50%H2O 40% Acetonitrile and 10% 100 mM ammonium Bicarbonate, pH 10. **Sample buffer** will consist of 38% Acetonitrile and 62% 10 mM ammonium acetate, pH 4.8 with 30 minute run time and a sample of mobile phase injected every tenth sample.

D. 1. b. iii. Stability of Risperidone and PLGA polymers during implant fabrication: The purpose of these experiments will be to demonstrate that neither risperidone nor selected PLGA polymers are altered by the implant fabrication process. We do not anticipate that the structure of these materials will be altered by dissolution in acetone, solvent casting and slow extrusion molding. However, this hypothesis will need to be proven to ensure the FDA that the final pharmaceutical compound does not differ from the currently approved formulation of risperidone.

D. 1. b. iii. a. Risperidone: Risperidone implants composed of 5% drug and 95% polymer (wt./wt.) will be made by solvent casting from acetone followed by slow extrusion molding into rods of 3.6 mm diameter at 60°C, 5 mm per minute piston speed (High Pressure Piston Extruder, DACA Instruments, Goleta, CA). The resulting material will undergo quantitative in-vitro analysis with HPLC for risperidone content. Drug content will be determined by dissolving implants in Acetonitrile. Aliquots of the resulting solution containing acetonitrile, risperidone and polymer will then be used to make sample buffer. Sample buffer will be centrifuged at 10,000 rpm to remove precipitated polymer. Supernatant, containing soluble risperidone and polymer, will then be injected into the HPLC for quantitative assessment of drug content. The resulting measurement will be expressed as a ratio of original mass of risperidone in the implant material.

D. 1. b. iii. b. Polymers: Stability of PLGA polymers will be assessed following fabrication procedures. Control implants will be prepared without drug using solvent casting and extrusion molding. The resulting material (5-10 mg) will be assessed for alterations in inherent viscosity in acetone using an Ubbelhode suspended-level viscometer (Fisher Scientific) at constant temperature (25°C). Values for inherent viscosity of each polymer prior to and following implant fabrication will be compared to assess if there has been any alteration in the size of the polymer molecules. Each material (10-15 mg) will also be assessed for its glass transition temperature (Tg) using differential scanning calorimetry between 20 and 100°C at 10°C/min (Perkin Elmer DSC-7, Wellesley, MA). As with inherent viscosity, polymer Tg will be determine prior to and following implant fabrication.

D. 1. b. iv. ***Stability of Risperidone-PLGA implants during storage:*** The purpose of these studies is to determine the stability of both risperidone and PLGA polymers during implant storage (shelf life). Stability of risperidone following incorporation into implants will be assessed by creating a set of 12 implants for each individual polymer (5% risperidone / 95% polymer) by solvent casting and extrusion molding. This will create a total of 120 implants (10 polymers x 12 replicates of each). All implants will be individually wrapped and stored in a desiccated environment at 4°C for various intervals. Four sets of three implants will then be placed into *in-vitro* assay systems following intervals of 1 week, 3 months, 6 months and 12 months of storage. This will determine the effects long-term storage of implants on both the maximum level of risperidone released as well as release profile (interval and pattern). The former (maximum amount) will be primarily dependent on the stability of risperidone, while the latter (release interval and pattern) will be determined by both the stability of the polymer and drug. The stability of each polymer will be assessed from a set of 120 control implants (no drug) consisting of 12 implants from each of the ten polymers. Groups of three blank implants from each material will be tested at 1 week, 3 months, 6 months and 1 year using inherent viscosity and glass transition analyses.

D. 1. c. Interpretation and Limitations:

D. 1. c. i. Stability of risperidone during in-vitro conditions: Preliminary data indicate that risperidone is stable for greater than 10 months using UV spectrophotometry (page 8). Additionally, risperidone is stable at room temperature for greater than 3 years in powder form (data provided by RBI, Flanders NJ). Therefore, we anticipate that risperidone implants will yield consistent mass of drug following the four proposed storage intervals. If there are significant reductions in the mass of risperidone following storage for 6 months, alternative methods including storage under nitrogen and storage at colder temperatures (-20°C and -80°C) will be investigated.

***D. 1. c. ii. Stability of polymers and drugs during fabrication*:** Processing of implant material will include dissolution in acetone followed by solvent casting and slow extrusion at 60°C. Dissolution in acetone is not expected to alter the chemical bonds of either the drug or polymers. However, if there is evidence of degradation during the solvent casting stage, other class 3 solvents (Table 2) in which both polymer and drug are soluble would be identified and tested as alternatives. Similarly, we do not anticipate that extrusion at 60°C will alter the molecular structure of either the drug or polymer as this is far below the melting temperature for risperidone (169°C) and all selected polymers can be processed at temperatures up to 140°C without degradation (data provided by Alkermes). However, if degradation of any material occurs at 60°C, alternative polymers will be identified from Alkermes and/or other medical grade polymer manufacturers to provide release within the desired intervals.

Table 2: FDA Class III solvents (FDA Guidance document Q3C)

Acetic acid	Cumene	Heptane	2-Methyl-1-propanol
Acetone	Dimethyl sulfoxide	Isobutyl acetate	Pentane
Anisole	Ethanol	Isopropyl acetate	1-Pentanol
1-Butanol	Ethyl acetate	Methyl acetate	1-Propanol
2-Butanol	Ethyl ether	3-Methyl-1-butanol	2-Propanol
Butyl acetate	Ethyl formate	Methylethyl ketone	Propyl acetate
tert-Butylmethyl ether	Formic acid	Methylisobutyl ketone	

D. 1. c. iii. Stability of polymers and drug during storage: Biodegradable polymers are expected to remain stable for greater than 12 months under appropriate storage conditions. Preliminary data indicate that implants yield highly consistent release interval and amount when used within 1 month of fabrication. The proposed studies will extend this knowledge and address a key logistic variable as real world applications of this technology will require storage of implants for longer periods when they are transported from manufacturing sites to patient locations. If risperidone-PLGA implants prove unstable over 6 months for any single polymer, alternative methods including storage under nitrogen and storage at colder temperatures (-20°C and -80°C) will be investigated. If these modifications do not yield sufficient stability, polymers with higher molecular weight and different end group capping would be tested.

D. 2. Aim 2: To determine the *in vivo* release profile for risperidone as a function of lactide to glycolide ratio and polymer inherent viscosity. Rationale: Drug release from biodegradable PLGA implants will be influenced by a series of reactions that vary for each drug-polymer combination. These reactions include hydration of implants, diffusion of drug from the implant into the surrounding aqueous environment and degradation of the polymer matrix. At the inception of release, drug molecules are homogenously interspersed with molecules of polymer to form a composite matrix of drug and polymer. The resulting composite is shaped into a macroscopic implant that shares physical characteristics from both constituents. During the initial release period, drug is physically constrained within this composite, and release is limited by surface diffusion. However, as water permeates the implant, the composite becomes hydrated, allowing for diffusion of drug across a concentration gradient as well as hydrolysis of polymer chains. As polymer chains become hydrolyzed, two processes are accelerated to facilitate movement of drug from the implant to the surrounding environment. First, water permeability increases as the polymer chains decrease in length. Secondly, molecules of drug are released from the physical entrapment. Since implants are composed of a high proportion of both drug and polymer, both materials will contribute to the speed of hydration and thus diffusion of drug and degradation of polymer. Drug concentration will not vary in the current proposal. All implants will have the maximal risperidone load possible, resulting in the smallest and most easily tolerated implants.

D. 2. a. Design:

D. 2. a. i. Release patterns for individual polymers: Preliminary data using haloperidol implants indicate that a single polymer system using 100% PLA results in a Gaussian release profile with peak concentration at 6 months and within the therapeutic range between 2 and 8 months post implantation (Figure 2a, page 7). As further shown in Figure 6, (page 10) this symmetrical release profile could be used to provide long term delivery by introducing a new set of implants every 6 months. Such a system would provide steady state long term delivery because each subsequent set of implants would increase medication release at the same rate that the previous system is declining. Although a simple, single polymer system presents many beneficial qualities, the major limitations result from the long lag to reach therapeutic levels following the initial implantation and relatively large oscillations around steady state following subsequent implantations. Therefore, we propose to use additional polymers that provide a more rapid time to peak concentration during initial implantation and to create tighter oscillations around a target concentration (Figure 7, page 16). Each implant type will then be tested in rat to determine the length and pattern of release *in-vivo*. The ten initial polymers for testing are listed in Table 1, page 12.

D. 2. a. ii. Gaussian release profiles: All release curves are expressed using the probability mass function of a normal distribution centered on a desired maximum (μ). The width and height of each curve are designated by the standard deviation (σ) and amplitude (α). The value of σ was set to provide full release within each desired interval such that values at both time of implantation and termination approach zero. The value of amplitude (α) for each curve was designated to allow oscillations around a value of 1.0 - 1.2 arbitrary concentration units (Figure 7).

Figure 7: Representation of an ideal multiple polymer-risperidone implant system.

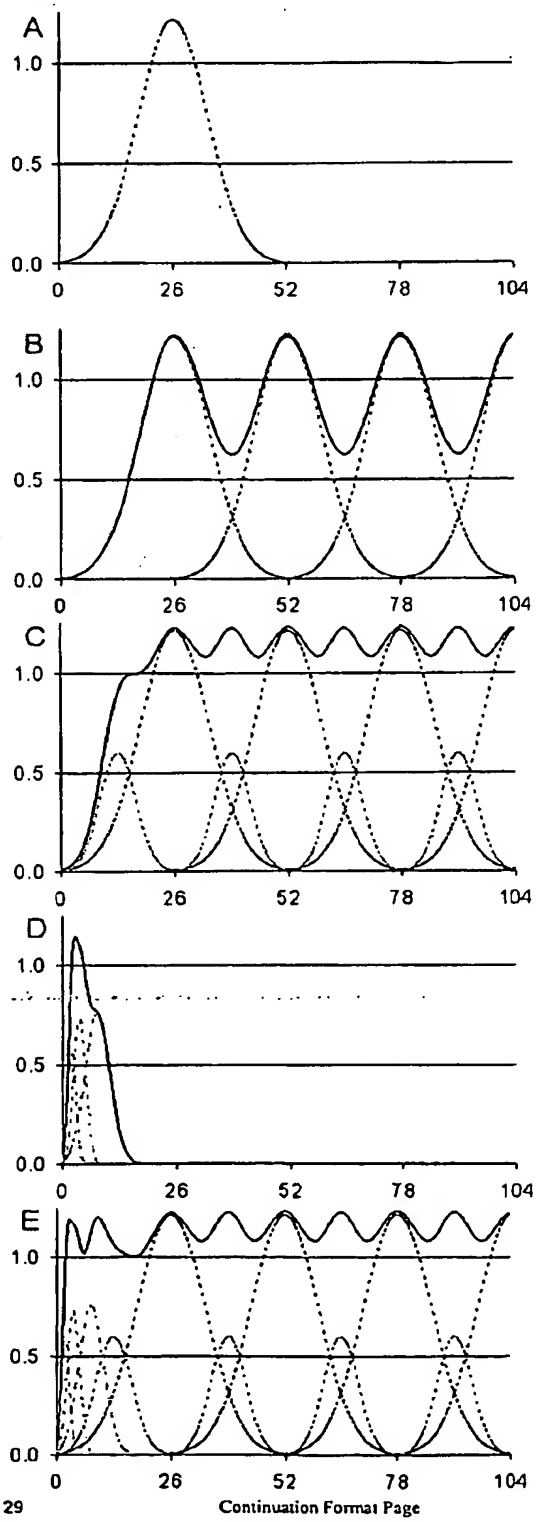
(A) Expected serum profile from a single polymer with maximum release (μ) at 6 months, full release at 12 months and maximum concentration of 1.2 units. The shape and duration of delivery are modeled on preliminary data using haloperidol from 100% PLA with 50% drug load (Figure 2, page 7).

B) Representation of combined release (dark solid line) following reimplantation of a single polymer every six months (dotted lines). Note that this system would result in a gradual escalation to therapeutic steady state during the first six months, followed by large oscillations above and below the desired serum concentration.

C) Demonstrates the pattern of release that would result from the addition of a second polymer with maximum release (μ) at 3 months and full release over 6 months. Note that the cumulative release pattern resulting from this 2-polymer "maintenance set" would gradually reach the desired level of 1.0 - 1.2 over approximately 4 months with smaller oscillations around the desired serum concentration thereafter.

D) Demonstrates the combined release (solid black line) resulting from a series of three "starter" polymers (gray dotted lines) that could be used at the first implantation surgery to bridge the 4-month lag that would exist from a 2-polymer system shown in panel C. Three individual polymers would achieve maximum release (μ) at 2.3, 4 and 8 weeks respectively. Amplitude (α) and standard deviation (σ) have been set so that each release curve has a value that approaches zero at onset and termination at 5, 8 and 16 weeks respectively.

E) Representation of 5-polymer system in which three rapidly degrading polymers (starter set) are combined with two longer lasting polymers (maintenance set) that are reimplanted every six months. Levels that result from all five individual polymers are represented with the solid line and release profiles from individual polymers are represented in gray dashed lines. Note that such a system could reach therapeutic target levels within 2.5 weeks of implantation with small oscillations around a designated concentration thereafter.



D. 2. b. Methods:

D. 2. b. i. Fabrication: Individual polymers and risperidone will be combined as previously described using solvent casting from acetone and slow extrusion molding into rods of 3.6 mm diameter (page 13). The length of implants will be determined by the dose required in each condition listed below. Extrusion molding will be done in a dedicated clean room with negative pressure ante room to ensure a positive pressure environment relative to the adjacent area. Implants will be individually wrapped and stored under vacuum in a desiccated chamber at 4°C.

D. 2. b. ii. Implantation: Rats (n = 40) will be anesthetized with isolfurane 5% induction, 1% maintenance with adjustments to maintain a surgical plane of anesthesia. The dorsal surface will be shaved and sterilized with betadine. A 4 mm skin incision will be made and a series of rods will be placed in the subcutaneous space. Each rod will be 3.6 mm in diameter with a density of 1.22 g/cc yielding rods with approximately 125 mg/cm of implant. Each animal will be given a length of implant to achieve an average dose of 2 mg/kg/day. No implant will be longer that 2 cm to ensure comfort of the animal. **Experimental conditions**: Implants made with each of 10 polymers (Table 1, page 25) will be combined with risperidone and placed in 4 rats per group.

D. 2. b. iii. Pharmacokinetic serum analyses: Two ml of blood will be taken from the tail vein for each of the first two weeks and then biweekly thereafter. Blood will be centrifuged and serum frozen at -80°C until analysis. Risperidone serum concentrations will be determined at each time point for each animal following solid phase extraction (MCX, Waters) and HPLC/UV detection as previously described. The *in-vivo* release profile for each material will then be determined by plotting serum concentration across time for each group of animals.

D. 2. c. Expected outcomes: Anticipated release intervals for risperidone from each polymer material are shown in Table 3. The expected period to maximum and full release are noted. Additionally, anticipated rates of release for risperidone from each polymer are noted. Based on these calculations, the proposed length of implant that is required to deliver 2 mg/kg/day for a 300 gram rat is listed.

Table 3: Expected outcome for implants from selected polymers and 60% risperidone load. All values are based on a target does of 2 mg/kg/day for a 300 g rat.

Polymer	max release (days)	full interval (days)	mg implant	cm implant
50:50 L	20	40	40	0.3
50:50 H	28	55	55	0.4
65:35 L	45	90	90	0.7
65:35 H	55	110	110	0.9
75:25 L	60	120	120	1.0
75:25 H	70	140	140	1.1
85:15 L	75	150	150	1.2
85:15 H	90	180	180	1.4
100:0 L	175	350	350	2.8
100:0 H	190	380	380	3.0

D. 2. d. Interpretation and Limitations

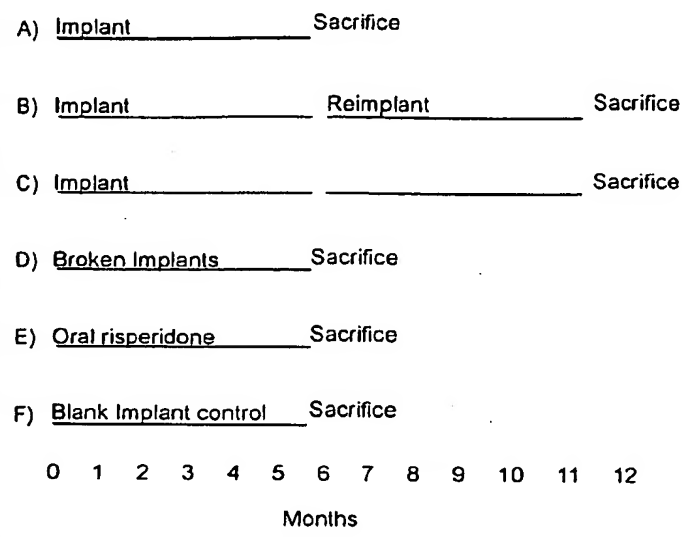
D. 2. d. i. Scalability to human: One potential concern with the proposed design relates to scalability of dose from rat to human. The current recommended human dose for parenteral risperidone from PLGA microspheres (Risperidone Consta®, Janssen Pharmaceutica) is 25-50 mg every 2 weeks. This yields a dose of 1.8 mg/day for the 25 mg injection. A similar dosing strategy from implants would require 325 mg of risperidone every six months. A six month implant system with 60% risperidone would therefore have a mass of 540 gm. At a density of 1.22 g per cc and diameter of 3.6 mm, this would require a total of less than 4.5 cm of implant rods. For example, the "maintenance" set of implants could be supplied as two rods of 2.25 cm length containing 100% PLA and one rod of 1.1 cm containing 85:15 PLGA. Additionally, a "starter" set could contain three implants, each 1.1 cm in length, composed of 50:50, 65:35 and 75:25 PLGA respectively. Thus, an annual plan of care could include four implants of 1.1 cm length and two implants of 2.25 cm length at initial implantation with three additional maintenance implants every six months.

D. 2. d. ii. Additional polymers: Studies outlined in Aim 2 will provide *in-vivo* pharmacokinetic data to create a composite system to deliver risperidone at steady state with reimplantation every 6 months. Data obtained with the ten initial polymers will be compared with the model outlined in Figure 7 (page 16) to determine if a suitable combination of polymers has been identified to provide rapid onset and continuous release of risperidone within the therapeutic range. However, if data indicate that the ideal combination of polymers has not been identified, additional polymers will be chosen based on the degree and direction of deviation from ideal. For example, if the selected 50:50 PLGA polymers yield release that is longer than the anticipated interval of 4 - 5 weeks, a 50:50 PLGA polymer with lower inherent viscosity could be tested.

D. 3. Aim 3: To assess local tissue and systemic toxicity for a semiannual formulation of implantable PLGA-risperidone implants. Rationale: The purpose of this Aim is to evaluate the toxicological effects of continuous release of risperidone from a implantable PLGA delivery system. Following selection of polymers in Aim 2, this resulting composite system will need to demonstrate safe and consistent release in one rodent and one non-rodent species prior to moving to phase-I human trials. Therefore, the final aim will demonstrate a lack of local tissue irritation and systemic toxicity following chronic *in-vivo* risperidone release from PLGA implants in rat and dog.

D. 3. a. Design: ***Serum kinetics and histopathology of composite implant system:*** Three doses of the combined PLGA-risperidone implant system derived in Aim 2 will be placed in the subcutaneous space in rats and dogs. Groups of animals will be sacrificed at either 6 months or 12 months for histopathology. Blood will be taken at 2-week intervals for determination of serum risperidone levels and monthly for hematology and serum chemistry in one rodent (rat) and one non-rodent (dog) species. These animals will also undergo weekly behavioral observation during the release interval. A subset of animals will be sacrificed for histopathologic evaluation at 6 months. The remainder will complete 12 months of drug delivery prior to necropsy and histopathology as outlined in Figure 8.

Figure 8: Experimental groups for serum pharmacokinetics and histopathology.



A) The first group will receive 6 months of exposure to risperidone from a multiple polymer system prior to sacrifice.

B) The second series of animals will receive an initial multi-polymer system followed by reimplantation of "maintenance" implants at six months to provide 12 months of continuous medication prior to necropsy.

C) The third group will receive an initial multi-polymer system to provide six months of steady state delivery, but will not receive reimplantation of maintenance implants. These animals will then be sacrificed at 12 months post implantation to provide toxicological evaluation after the system has ceased to delivery medication.

D) The fourth group of rats will receive broken implants to evaluate the consequences of mechanical damage to implants during the delivery interval.

E) The fifth group will serve as positive control animals and will receive daily oral risperidone (dog) or risperidone injections (rat) to reveal toxicological effects of the drug that are independent of the delivery system being tested.

F) The final group of animals will receive blank implants with no drug as a negative control to assess the effects of polymer materials, independent of risperidone.

D. 3. b. Methods

D. 3. b. i. Materials and Implant Fabrication: Risperidone and Medisorb polymers will be obtained from the NIMH synthesis program and Alkermes respectively as described in Aims 1 and 2. Implants for all toxicological studies will be fabricated by solvent casting and extrusion molding as previously described in a dedicated clean environment.

D. 3. b. ii. Implantation: There will be 4 animals per experimental condition (2 male, 2 female). **Rats** (n = 48) and **Beagles** (n=28) will be anesthetized with isolfurane 5% induction, 1% maintenance with adjustments to maintain a surgical plane of anesthesia. The implantation site will be shaved and sterilized with betadine. A 4 mm incision will then be made and 6 rods will be placed in the subcutaneous space. We will also address the issue of variable absorption from different body locations. Two animals will receive implants on the dorsal surface of the back, while two others will receive implants on the lateral surface of the rear leg.

D. 3. b. iii. Phlebotomy: Two ml of blood will be taken from each rat (0.5% body weight) and 15 ml of blood will be taken from each dog (0.25% body weight) for pharmacokinetic analyses and hematology/serum chemistry. These volumes will allow for each rat sample to be used for either serum risperidone or hematology/serum chemistry, while the larger volume from dog will allow for determination of both risperidone level and hematology/chemistry from a single sample. Therefore, dogs will have blood drawn each

of the first two weeks and biweekly thereafter, yielding two serum risperidone and one hematology/serum chemistry sample per month. Rats will have three samples per month taken to allow for two serum risperidone and one hematology/serum chemistry samples per month.

D. 3. b. iv. Serum Hematology and chemistries: One sample per month will be split for hematology (1 ml) and serum chemistry (1 ml), which will include: 1) complete blood count (CBC) with differential (Hg, Hct, WBC, and platelets); 2) serum electrolytes (sodium, potassium, calcium, chloride, $C0_2$, blood urea nitrogen (BUN), and creatinine); 3) liver function tests (LFTs, ALT, GGT and Alkaline phosphatase). All chemistry and hematology will be performed by an accredited GLP clinical laboratory.

D. 3. b. v. Serum pharmacokinetics: Serum risperidone level will be performed using solid phase extraction and HPLC/UV detection as described (page 12) using Breeze software (Waters). Each sample will consist of 1.0 cc of serum and will be run in duplicate. All data will be collected on a secure computer. Data will be backed up daily to a secure server and copied to tape monthly. Hard copies of all chromatographs will also be stored in a secure location.

Table 4: Rat studies will use 4 animals per condition. Doses are in mg/kg/day

Condition	Dose	Description
Neg. Control	0.0	Single implantation, necropsy at 6 months
Pos. Control	1.5	Daily injections of 1.5 mg/kg
dose 1	0.5	Single implantation, necropsy at 6 months (peak value)
dose 1	0.5	Single implantation, necropsy at 12 months (after system runs out)
dose 1	0.5	Initial implant & reimplant at 6 mo, necropsy at 12 mo (peak value)
dose 2	1.0	Single implantation, necropsy at 6 months (peak value)
dose 2	1.0	Single implantation, necropsy at 12 months (after system runs out)
dose 2	1.0	Initial implant & reimplant at 6 mo, necropsy at 12 mo (peak value)
dose 3	1.5	Single implantation, necropsy at 6 months (peak value)
dose 3	1.5	Single implantation, necropsy at 12 months (after system runs out)
dose 3	1.5	Initial implant & reimplant at 6 mo, necropsy at 12 mo (peak value)
dose 3 (broken)	1.5	Single implantation, necropsy at 6 months (peak value)

Table 5: Dog studies will use 4 animals per condition. Doses are in mg/kg/day

Condition	Dose	Condition
Neg. Control	0	Single implantation, necropsy at 6 months
Pos. Control	1.5	Daily oral administration of risperidone
Dose 1	0.5	Single implantation, necropsy at 6 months (peak value)
dose 2	1.0	Single implantation, necropsy at 6 months (peak value)
dose 3	1.5	Single implantation, necropsy at 6 months (peak value)
dose 3	1.5	Single implantation, necropsy at 12 months (after system runs out)
dose 3	1.5	Initial implant & reimplant at 6 mo, necropsy at 12 mo (peak value)

D. 3. b. vi. Pathology: Animals will be sacrificed by overdose with sodium pentobarbital under the supervision of a University Laboratory Animal Resources (ULAR) veterinary technician at the end of each test interval. Necropsy will be performed by Ronnie Cimprich, VMD, who is an accredited veterinary pathologist (letter of credentials included). Dr. Cimprich has worked with the principal investigator in the past and performed necropsy for the previous series of rabbits that received haloperidol implants. Following necropsy, all samples will be sent to American Histolabs, which is an accredited GLP histological service, for preparation of microscopic samples for haematoxylin and eosin slides. Slides will then be returned directly to Dr. Cimprich for interpretation and documentation. The following tissue types will be evaluated:

> Adrenal, Artery (aorta), Bone (femur), Bone Marrow (sternum), Brain, Cecum, Colon, Duodenum, Epididymis, Esophagus, Eye/optic nerve, Gall bladder, Heart, Ileum, Implant sites, Jejunum, Kidney, Liver, Lung, Lymph node (mesenteric), Lymph node (submaxillary), Mammary gland, Muscle, skeletal, Nerve, sciatic, Ovary, Pancreas, Pituitary, Prostate, Salivary Gland, Skin, Spinal cord, Spleen, Stomach, Testis, Thymus, Thyroid/Parathyroid, Trachea, Urinary bladder, Uterus, and Vagina.

D. 3. c. Interpretation and Limitations: There are two main objectives for the final Aim of this proposal. First, we will test the ability of our composite polymer design to deliver 6 or 12 months of continuous risperidone following a single or repeated implantation scheme, respectively. Secondly, we will determine whether or not local or systemic toxicity is introduced by this novel delivery system in comparison to the effects of risperidone alone.

D. 3. c. i. Pharmacokinetics: One potential limitation to our approach is the use of Gaussian equations to model release from biodegradable PLGA implants. This approach was chosen for two reasons. The first reason is that *in-vivo* release of haloperidol from 100% PLA approximates a Gaussian shape (Figure 2a, page 7). Additionally, *in-vitro* cumulative release curves for many agents, including haloperidol and risperidone, approximate the shape of the cumulative normal distribution (Siegel et al., 2002) and (Figures 5, 6, page 9). Therefore, we believe that this model provides a useful framework in which to design an ideal long term delivery system using a combination of polymers. Although the use of symmetric distributions is a useful illustration of the concept of combining various polymers to achieve steady state, the true release profiles *in-vivo* will differ slightly from these ideal curves. Specifically, release from biodegradable PLGA implants may be asymmetric and/or have periods of relatively high dosing early in the release period due to initial surface diffusion. However, it is important to note that the final composite system in Aim 3 will be based on actual *in-vivo* release profiles generated for each polymer-risperidone composite material in Aim 2. Therefore, we believe that we will be able to incorporate asymmetries or periods of increased release to formulate a final combination that yields steady extended release. For example, increased early release from each polymer could negate the need for an implant that releases risperidone over the first 4-5 weeks. Alternatively, if it is not possible to achieve rapid onset of therapeutic levels within 2-3 weeks using PLGA implants, patients could receive an injection of the existing formulation of risperidone microspheres (Risperidone Consta, Janssen Pharmaceutica) to bridge this initial period.

D. 3. c. ii. Pathology: We do not anticipate local toxicity or systemic effects that differ from risperidone alone based on histopathologic data with haloperidol-PLGA implants in rabbit. Specifically, a series of previous studies has allowed our group to modify implant design and implantation procedures to minimize or abolish local tissue reactions. If any material is recovered from the implant locations as occurred in rabbits, it will be analyzed for both drug and polymer content using HPLC and NMR. We do expect to replicate any systemic effects of risperidone alone using implants. These are likely to include mild, reversible hepatotoxicity, as the active drug is metabolized in the liver and is known to increase liver enzymes in clinical doses. Since this program is directed at the delivery system rather than the pharmaceutical compound, we do not anticipate that toxicity that is equal to or less than oral administration would be a contraindication to either FDA approval or clinical application in humans.

D. 4. Future Directions

The goal of this application is to complete preclinical studies that will enable implantable antipsychotic delivery systems to proceed to phase I clinical trials in humans. Although studies within this application are designed without the need for direct involvement of industry, the investigators recognize the need to involve an industrial partner to move to human. All technology and intellectual property within this application has been disclosed to The University of Pennsylvania Center for Technology Transfer (CTT), which is actively engaged in moving translational products from the academic setting to industry. A letter of support is enclosed from Lewis Bernaman, Director of CTT, describing the University's commitment to moving this technology into industry through the pursuit of patents, licensing and venture capital. Additionally, the PI has worked closely with the Office of Human Research at the University of Pennsylvania over the past year in preparation for eventual transition to human studies. A letter of support from Dr. Gregg Fromell, Director of the Office for Human Research at Penn, describes the commitment of his office to helping the investigators move towards human trials. In summary, the investigators and University of Pennsylvania are committed to the idea that implantable delivery systems will improve schizophrenia treatment and will dedicate the resources to keep this effort moving towards humans in collaboration with industrial partners.

E. Human Subjects – None

F. Vertebrate Animals: Institutional Animal Care and Uses Committee (IACUC) at the University of Pennsylvania approved all protocols and animals will be housed in AAALAC accredited animal facilities. All animals will be obtained from Harlan (Indianapolis, IN). Each experimental group will consist of four animals. This number was determined under advisement of the psychopharmacology team at the Food an Drug Administration during discussions directed towards designing preclinical studies that would be reviewed prior to phase I application. Experimental conditions for Aim 2 will utilize a total of 40 rats. If the initial set of 10 polymers chosen do not yield appropriate release patterns and intervals, then additional polymers will be tested. Each additional polymer will require an additional 4 rats. Aim 3 will utilize a total of 48 rats and 28 dogs as specified in tables 4 and 5 respectively (page 22). As with Aim 2, these numbers reflect the initial conditions and anticipated outcomes. If however, there are periods of excess or diminished release relative to target range, additional experimental groups will be needed and would be added with 4 animals per group. In such an event, a justification for additional animals would be filed with IACUC and NIH would be informed.

Description of procedures employing animals: Please see preliminary studies and methods sections for detailed descriptions of all animal protocols.

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